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Drug Monitoring and Toxicology

Stereospecific Analysis and Enantiomeric Disposition of 3,4-Methylenedioxymethamphetamine (Ecstasy) in Humans

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Background: Little is known concerning the enantioselective disposition of 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) in humans. In addition, the potential of utilizing the stereochemical composition of an analyte in biological media for forensic purposes requires investigation.

Methods: The enantiomers of MDMA and its demethylated metabolite, 3,4-methylenedioxyamphetamine (MDA), present in plasma and urine extracts were derivatized with (−)-(*R*)-α-methoxy-α-trifluoromethylphenylacetyl chloride and analyzed by gas chromatography–mass spectrometry and gas chromatography, respectively. The enantioselective disposition of MDMA and MDA was determined following oral administration of racemic MDMA (40 mg) to eight male volunteers.

Results: The plasma concentrations of (*R*)-MDMA exceeded those of the *S*-enantiomer [ratio *R*:*S* of the area under the curve (AUC), 2.4 ± 0.3], and the plasma half-life of (*R*)-MDMA (5.8 ± 2.2 h) was significantly longer than that of the *S*-enantiomer (3.6 ± 0.9 h). The majority of the recovered material in urine was excreted within 24 h after dosing, with the recovery of (*R*)-MDMA (21.4% ± 11.6%) being significantly greater than that of (*S*)-MDMA (9.3% ± 4.9%), and with (*S*)- and (*R*)-MDA accounting for 1.4% ± 0.5% and 1.0% ± 0.3% of the dose, respectively. Mathematical modeling of plasma enantiomeric composition vs sampling time demonstrated the applicability of using stereochemical data for the prediction of time elapsed after drug administration.

Conclusions: Analytical methods for determining the enantiomeric composition of MDMA and MDA in plasma and urine were developed. The disposition of MDMA in humans is stereoselective, with the more active *S*-enantiomer having a reduced AUC and shorter half-life than (*R*)-MDMA. The determination of stereochemical composition may be applicable for forensic purposes.

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3,4-Methylenedioxymethamphetamine (MDMA)[4] is a commonly used recreational drug known as "ecstasy", "E", or "Adam". In contrast to methamphetamine, it has a methylenedioxy substituent on the phenyl ring, which results in distinctive psychoactive properties *(1)*. 3,4-Methylenedioxyamphetamine (MDA), itself a popular drug of abuse in the 1960s [known as the "Love Drug" in the US *(2)*], is the N-demethylated metabolite of MDMA *(3)*. Although they have different overall effects, both drugs are known to cause changes in mood and perception and to produce feelings of euphoria and empathy *(4, 5)*. The widespread recreational use of MDMA in both the UK *(6)* and US *(7)* has been associated with problems

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[4] Nonstandard abbreviations: MDMA, 3,4-methylenedioxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; HMMA, 4-hydroxy-3-methoxymethamphetamine; GC, gas chromatography; MTP chloride, α-methoxy-α-trifluoromethylphenylacetyl chloride; HP, Hewlett-Packard; AUC, area under the plasma concentration-time curve; MS, mass spectrometry; C_{max}, maximum observed plasma concentration; t_{max}, time to attain maximum observed plasma concentration; $t_{1/2}$, elimination half-life; CL, clearance; F, systemic availability; V_D, volume of distribution; CL_R, renal clearance; HCDA, homochiral derivatizing agent; and *Rs*, chromatographic resolution factor.

of toxicity ranging from abnormalities of water homeostasis *(8)* to renal and hepatic failure *(9)*, and fatalities have been reported *(9, 10)*. MDMA may also cause serotonergic neurotoxicity, the potential long-term effects of which may not be known for years *(11)*.

MDMA is a chiral compound used as a racemate, and an examination of the pharmacological activity of the individual enantiomers indicates that the (+)-*S*-enantiomer is more active in terms of "the degree and the disruptiveness of the induced intoxication" *(12)* than the (−)-*R*-enantiomer. Studies in animals have indicated that the drug also undergoes stereoselective disposition, with the (+)-*S*-enantiomer having a shorter half-life than the (−)-*R*-enantiomer in the rat *(13)*. There are few reports concerning the enantioselective disposition of MDMA in humans. Moore et al. *(14)* examined the distribution of the enantiomers of MDMA and MDA in bile, blood, liver, urine, and vitreous humor following a fatal poisoning from insufflation of MDMA, cocaine, and heroin. The data obtained indicated that the disposition of MDMA in humans is stereoselective, with the concentrations of (−)-(*R*)-MDMA exceeding those of the *S*-enantiomer in all tissues and fluids examined. In contrast, the concentrations of (+)-(*S*)-MDA were greater than those of the *R*-enantiomer in bile and urine and were approximately equal in the blood and liver, whereas the concentration of (−)-(*R*)-MDA was greater than that of the *S*-enantiomer in the vitreous humor. De Boer et al. *(15)* analyzed human urine samples obtained from three unrelated MDMA intoxications and reported preliminary results indicating enantioselective urinary recovery of MDMA and MDA. However, because of the lack of the individual enantiomers, the stereochemical composition of the material could not be assigned. Lanz et al. *(16)* reported 72-h urinary excretion profiles for MDMA, MDA, and 4-hydroxy-3-methoxymethamphetamine (HMMA) enantiomers in two subjects following administration of 1.5 mg/kg body weight of racemic MDMA. The urinary recovery of (−)-(*R*)-MDMA was between three- to fourfold greater than that of the *S*-enantiomer, and the urinary enantiomeric composition of both MDA and HMMA showed variable stereoselectivity both with time and between subjects. Again, the lack of stereochemically defined authentic standards precluded data interpretation.

To the best of our knowledge, the pharmacokinetic properties of the enantiomers of MDMA in humans have not been reported in the literature. This is in part because of the legal and ethical problems associated with the administration of a Schedule 1 controlled drug and specifically the difficulties in obtaining enantiomerically pure reference compounds for both MDMA and MDA. We have developed enantiospecific methods based on capillary gas chromatography (GC) for the determination of MDMA in plasma and urine. Because these methods can be applied equally without modification to the measurement of MDA, we chose to analyze the enantiomers of both compounds following oral administration of racemic MDMA, although MDA does not appear to be the major urinary metabolite of MDMA *(16, 17)*. Here, we report the enantioselective disposition of MDMA in humans, together with the potential utility of the determination of stereochemical composition in forensic science.

Materials and Methods

MATERIALS

Analytical grade hexane, ethyl acetate, methanol, sodium chloride, and potassium hydroxide were purchased from Fisher Scientific UK; (±)-(*R*,*S*)-MDMA hydrochloride, (±)-(*R*,*S*)-MDA hydrochloride, (±)-(*R*,*S*)-amphetamine sulfate, (−)-(*R*)-amphetamine base, and (+)-(*S*)-amphetamine sulfate were purchased from the Sigma Chemical Co. (−)-(*R*)- and (+)-(*S*)-MDMA hydrochloride and (−)-(*R*)- and (+)-(*S*)-MDA hydrochloride were generously donated by the Research Technology Branch of the National Institute on Drug Abuse, Rockville, MD. (±)-(*R*,*S*)-Methoxyphenamine hydrochloride was purchased from Upjohn; (+)-(1*S*,2*S*)-pseudoephedrine was purchased from The Wellcome Research Laboratories and Sandoz Products; (−)-(*R*)-α-methoxy-α-trifluoromethylphenylacetyl chloride [(*R*)-MTP chloride] and (+)-(*S*)-α-methoxy-α-trifluoromethylphenylacetyl chloride [(*S*)-MTP chloride] were purchased from Aldrich Chemical Co. The enantiomeric purity of (*R*)-MTP chloride and the individual enantiomers of MDMA and MDA were determined as described below.

INSTRUMENTATION

Urinary drug and metabolite concentrations were determined by GC, using a Hewlett-Packard (HP) 5890A instrument equipped with a nitrogen phosphorous detector and a cross-linked 50% phenyl methyl silicone capillary column (DB17; length, 30 m; internal diameter, 0.25 mm; film thickness, 0.25 μm) obtained from J & W Scientific. Peaks were manually integrated, using HP 3365 Series II Chemstation software. Analysis of drug and metabolite concentrations in plasma was performed by GC–mass spectrometry (GC-MS) using a HP 5890 Series II Plus GC fitted with a cross-linked methyl silicone capillary column (HP Ultra 1; length, 25 m; internal diameter, 0.2 mm; film thickness, 0.11 μm) and coupled to a HP 5972 Series mass selective detector operated in the selected ion monitoring mode. Maximum peak intensity values for each ion were obtained manually after background subtraction using a HP G1034C, Ver. C.03.00, software package.

VOLUNTEER STUDY PROTOCOL

Eight healthy, non-drug-using male volunteers (ages, 22–32 years), of whom one was Asian and seven were Caucasian, participated in the study. Home Office permission was granted to administer MDMA, a class A drug in the UK under the Misuse of Drugs Act 1971, and ethical approval and volunteer informed written consent were obtained in accordance with our institutional procedures.

Each volunteer was in good general health and had a normal heart rate and blood pressure, and liver function tests (plasma aspartate transaminase, alanine transaminase, and γ-glutamyltransferase) were within reference intervals. No volunteer was receiving any drug treatment and had not recently been involved in any other study of a similar nature.

Subjects were required to abstain from alcoholic beverages for 24 h before and during the study, and normal water loading was maintained from 2200 on the night before the investigation. On the morning of the study, each subject was given 47.5 mg of (±)-(*R,S*)-MDMA hydrochloride (equivalent to 40 mg of MDMA base) in capsule form with ~200 mL of water at 1000, 2 h after a light caffeine-free breakfast.

Blood samples (20 mL) were collected from a cannulated forearm vein immediately before and at 0.5, 1, 2, 4, 6, 8, and 24 h post drug administration. The samples were collected into heparin-containing tubes and immediately centrifuged at 4 °C for 15 min, and the plasma was separated. Urine samples were collected before drug administration and then continuously at 0–2, 2–4, 4–6, 6–8, 8–12, 12–24, 24–48, and 48–72 h after drug administration. All plasma and urine samples were rapidly frozen using liquid nitrogen and stored at −20 °C until required for analysis.

Analysis of urine samples

Urinary calibrators (5 mL) containing racemic MDMA and MDA were prepared to give single enantiomer MDMA concentrations of 0.05, 0.2, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 mg/L and single enantiomer MDA concentrations 10-fold more dilute than those of MDMA.

An internal standard solution (50 μL), comprising racemic methoxyphenamine (400 mg/L) and amphetamine (200 mg/L) in methanol, was added to each 5 mL of the calibrator, volunteer, and quality-control [prepared in batches by adding aqueous (*R,S*)-MDMA and (*R,S*)-MDA to urine] samples. The urine pH was adjusted to pH 13–14 by the addition of potassium hydroxide solution (0.5 mL of a 5 mol/L solution), and the ionic strength was increased by the addition of sodium chloride (~0.5 g). We performed liquid/liquid extraction by adding 2 mL of n-hexane/ethyl acetate (1:1, by volume) to each sample, vortex-mixing the solution vigorously (10 s), and then mixing the solution more gently for 10 min on a rotary mixer. The mixture was then centrifuged for 5 min at 800*g* to ensure phase separation, and the organic phase was then transferred to a 2-mL glass vial. The derivatizing agent (*R*)-MTP chloride (33 mL/L) in hexane was added (20 μL), and the vial was sealed with a PTFE-lined aluminum crimp cap and heated at 80 °C for 20 min. After the derivatized extract was cooled to room temperature, 1 μL was injected into the GC.

The GC was operated in the splitless mode with an injection port temperature of 250 °C; the carrier gas used was nitrogen, with a flow rate of ~2 mL per min; the detector temperature was 300 °C. The initial column temperature was 50 °C for 2 min, and was increased by 25 °C/min to 250 °C and then by 2 °C/min to 290 °C for 2 min, the total run time being 30 min.

The first and second eluting methoxyphenamine-MTP diastereomeric amide derivative peaks were used as internal standards for the (*R*)- and (*S*)-MDMA-MTP amide derivatives, respectively, and the corresponding (*R*)- and (*S*)-amphetamine-MTP derivatives were used as internal standards for the (*R*)- and (*S*)-MDA-MTP diastereomeric amides. Calibration curves were constructed by plotting the ratio of the peak area of each derivatized analyte to its respective internal standard derivative (peak area ratio) against the concentration of each analyte enantiomer, using a weighted linear regression fit with weights, $w_i = 1/\Delta^2 y_i$, where $\Delta^2 y_i$ is the square of the difference between the observed peak area ratio and the fitted value (computer software, Multicalc Advanced, Ver. 1.24; Pharmacia LKB Biotechnology). The analyte concentrations in each volunteer and quality-control sample were obtained by interpolation from the appropriate curves.

Analysis of plasma samples

Plasma calibrators (2 mL) containing racemic MDMA and MDA were prepared to give single enantiomer MDMA concentrations of 0.5, 1, 2.5, 5, 10, 20, 30, 40, 50, 60, and 80 μg/L and single enantiomer MDA concentrations 10-fold more dilute than those of MDMA. Samples in which single enantiomer MDMA concentrations were <1 μg/L were re-analyzed using lower calibrator concentrations: 0.10, 0.25, 0.40, 0.50, 1.0, and 2.5 μg/L enantiomer concentrations. The corresponding MDA enantiomer concentrations were maintained at one-tenth of those of the MDMA.

Internal standard solution (50 μL) containing racemic methoxyphenamine (4 mg/L) and amphetamine (1 mg/L) in water was added to 2 mL of each calibrator, volunteer, and quality-control sample [prepared in batches by the addition of aqueous (*R,S*)-MDMA and (*R,S*)-MDA stock solutions to plasma]. The plasma pH was adjusted to pH 13–14 by the addition of potassium hydroxide solution (0.5 mL of a 5 mol/L solution), and the ionic strength was increased by the addition of sodium chloride (~0.25 g). The extraction procedure was identical to that used for the analysis of the urine samples except that the organic phase was transferred to a glass test tube after separation by centrifugation. *(R)*-MTP chloride in hexane (10 μL of a 33 mL/L solution) was added, and the tube was capped and sealed (using aluminum foil) and heated at 80 °C for 20 min. After derivatization, the organic phase was evaporated to dryness under nitrogen at 30 °C, the residue was reconstituted in 40 μL (20 μL for the <1 μg/L assay) of n-hexane/ethyl acetate (1:1, by volume), and 1 μL was injected into the GC-MS.

The GC was operated in the splitless mode with an injection port temperature of 250 °C. The carrier gas was helium, with an inlet pressure of 21 psi. The interface

temperature was 280 °C; the initial column temperature was 100 °C for 3 min, and was increased by 15 °C/min to 285 °C for 5 min, the total run time being 20.3 min. For identification purposes, the ions monitored for each diastereomeric derivative were as follows: amphetamine-MTP, *m/z* 119, 139, 162, 189, and 260; methoxyphenamine-MTP, *m/z* 139, 148, 189, 200, and 274; MDA-MTP, *m/z* 135, 162, 189, and 260; MDMA-MTP, *m/z* 135, 162, 189, 200, and 274. The common ion *m/z* 189 is a fragment associated with the MTP moiety. The diagnostic ions chosen for quantitative purposes were *m/z* 162 for MDMA, MDA, and amphetamine and *m/z* 148 for methoxyphenamine.

Calibration curves were constructed by plotting peak height ratios of the selected ions of each analyte derivative to those of the selected internal standard derivative against the concentration of each analyte enantiomer. Calibration curves of weighted linear regression fit were constructed using the same analyte-to-internal standard ratio combinations and the same calculation method and computer software as described above. Volunteer and quality-control sample analyte concentrations were interpolated from the respective curves.

VALIDATION OF ANALYTICAL METHODOLOGIES

Optical purity of derivatizing reagent (R)-MTP chloride. The optical purity of (*R*)-MTP chloride was determined by derivatizing optically pure (+)-(*1S*,*2S*)-pseudoephedrine and determining by GC the diastereomeric composition of the amide derivatives formed. The amide derivatives of (+)-(*1S*,*2S*)-pseudoephedrine prepared after derivatization with either (*R*)- or (*S*)-MTP chloride eluted with retention times of 21.3 and 20.8 min, respectively. The quantity of (*S*)-MTP chloride as an enantiomeric impurity in (*R*)-MTP chloride was expressed as a percentage of the total of both derivative peak areas.

Optical purity of the individual enantiomers of MDMA and MDA. The optical purity of the individual enantiomers of MDMA and MDA was determined by derivatizing the individual enantiomers with (*R*)-MTP chloride and measuring with GC (using peak areas) the amount of each diastereomeric derivative present.

Enantiomeric excess and quantification. To determine whether the analytical procedure could produce accurate data with respect to enantiomeric composition over a wide range of concentrations, we prepared a series of plasma and urine calibrators containing different concentrations and enantiomeric composition for each MDMA and MDA enantiomer. "Total" MDMA concentrations of 8, 4, and 2 mg/L in urine and 80, 20, and 8 μg/L in plasma were prepared with the following enantiomeric *R*:*S* compositions: 80:20, 60:40, 40:60, and 20:80 for each concentration. MDA concentrations in the same samples were prepared at one-tenth of those of the corresponding MDMA concentrations. Five samples for each ratio were prepared, and the concentrations of both analytes, in both media, were determined as described above.

Limit of quantification. The limit of quantification of each analyte in both analytical methodologies was determined as the lowest concentration in the calibration curve at which the mean analyte peak height-to-baseline noise ratio was 10.

Within- and between-assay accuracy and precision. Urine and plasma quality controls were analyzed to determine assay accuracy and precision for the MDMA and MDA enantiomers.

Extraction efficiency. Extraction efficiencies for both analytes in urine and higher concentrations in plasma were determined at two concentrations (n = 5), within the respective calibration ranges, using racemic MDMA and MDA. Derivatized n-hexane/ethyl acetate extracts, prepared using the plasma and urine assay methodologies, were compared with derivatized solvent (n-hexane/ethyl acetate) aliquots containing equivalent concentrations of the racemic analytes.

Parallelism (urine dilution recovery). The parallelism was assessed to allow the analysis of volunteer urine samples that had single enantiomer concentrations of MDMA exceeding the calibration range. Urine was supplemented with racemic MDMA and MDA so as to exceed the calibration range (enantiomeric concentrations, 12 mg/L MDMA and 1.2 mg/L MDA) and then diluted two-, three-, four-, and fivefold with drug-free urine. Aliquots (2 × 5 mL) of each were analyzed, and the percentage of observed/expected values was calculated.

TREATMENT OF DATA

The maximum observed drug and metabolite enantiomer plasma concentrations (C_{max}) and the time to attain them (t_{max}) were obtained from an examination of the individual data points. The MDMA enantiomer elimination half-lives ($t_{1/2}$) were calculated using the method of least squares from the terminal linear phase of the semilogarithmic plasma concentration vs time curves. At least three data points were used for each calculation, depending on the curve profile. Areas under the plasma enantiomer concentration-time curves (AUC_{0-24}) were estimated using the trapezoidal method up to 24 h post drug administration. The values of the AUC were extrapolated to time infinity ($AUC_{0-\infty}$), using C_{24}/k, where C_{24} is the plasma concentration at 24-h post drug administration and k is the terminal dispositional rate constant. The apparent oral clearance of each MDMA enantiomer ($CL/F = D/AUC_{0-\infty}$) and volume of distribution ($V_D/F = CL/k$) were calculated by considering the dose (D) to be equal to one-half the administered dose of the racemate, and F to be the systemic availability. Renal clearance (CL_R) was calculated from $CL_R = A_{24}/AUC_{0-24}$, where A_{24} is the

amount of material excreted in the urine over the 0- to 24-h collection period.

The potential utility of drug and/or metabolite enantiomeric composition in biological media for forensic applications was examined by multiple regression analysis using the SPSS software statistics package (SPSS UK; see *Results and Discussion*).

Results and Discussion

ANALYTICAL METHODOLOGY

Following derivatization of MDMA and MDA using (*R*)-MTP chloride as a homochiral derivatizing agent (HCDA), the diastereomeric amides formed were resolved using the chromatographic conditions used for the analysis of both plasma and urine samples (Figs. 1 and 2). The stereochemical purity of the HCDA, (*R*)-MTP chloride, was determined by derivatization of enantiomerically pure (+)-(*1S,2S*)-pseudoephedrine and examination of the diastereomeric composition of the derivatives, using the GC methodology. Under the experimental conditions used, the stereochemical purity of the reagent was determined to be >99.5% (stated purity, 98% enantiomeric excess). Throughout the course of the investigations, the reagent was stereochemically stable under the conditions of use and storage, and between-batch analysis indicated a stereochemical purity of >99.5% in all cases. To ease subsequent calculations, the purity of the reagent was assumed to be 100%. Using this approach, we evaluated the stereochemical purity of the individual analyte enantiomers and found it to be ~99% [enantiomeric impurity, 0.38% in (*S*)-MDMA, 0.12% in (*R*)-MDMA, 0.82% in (*S*)-MDA, and 1.13% in (*R*)-MDA].

The derivatization and chromatographic properties of several related chiral amines were investigated to select an appropriate internal standard for the analytical methodologies. To compensate for possible differences in the reactivity of the acylating agent with primary and secondary amines, differing detector responses toward the amide derivatives formed with each analyte, and the expected differences in biofluid concentrations of MDMA and MDA, we deemed it appropriate to select two internal standards. Following evaluation of several compounds, methoxyphenamine and amphetamine were selected as the internal standards for MDMA and MDA, respectively. The chromatographic elution order of the amide derivatives was determined by derivatization of the individual enantiomers, and the diastereoisomer of configuration *R*-amine-*S*-MTP eluted before that of *S*-amine-*S*-MTP in each of the three cases in which the individual analyte enantiomers were available, i.e., MDMA, MDA, and amphetamine. As pointed out above, the amines were derivatized using (*R*)-MTP chloride as a HCDA, but as a result of the sequence rules, the configurational designation of the chiral center in the acyl MTP moiety changed from *R*- in the acyl chloride to *S*- in the amide derivatives. In the case of methoxyphenamine, the individual enantiomers were not available, and the elution order is unknown. It is, however, likely that the order corresponds to that of the other compounds. Chromatograms of derivatized authentic standards, extracts of blank plasma and urine samples, together with extracts of plasma and urine obtained from a volunteer at various times post MDMA (40 mg) administration are shown in Fig. 1. Chromatograms obtained following isolation of the four analytes from "blank" supplemented plasma and a volunteer sample at or near the limit of quantification of MDA are presented in Fig. 2. Under the conditions used for the analysis of both plasma and urine samples, the chromatograms were free from endogenous substances at the retention times of the analyte derivative peaks, the overall run times for the two methodologies being 20.3 and 30 min for the analysis of plasma and urine samples, respectively. Chromatographic separation and resolution (*Rs*) factors were calculated for each pair of analyte peaks, using both methodologies. The separation factors varied between 1.008 and 1.034 for the derivatives of methoxyphenamine and MDA, respectively; when the urine sample methodology was used, the corresponding *Rs* values were 1.23 and 4.80. Similarly, the lowest and highest separation factors when the plasma sample method was used were 1.005 and 1.014 for methoxyphenamine and MDA, respectively, with *Rs* values of 1.62 and 4.22. The extraction recoveries of the analytes were 82% and 68% for MDMA and MDA, respectively, in urine and 90% and 82% for the same compounds in plasma (all values being the means of data derived from the analysis of two sample concentrations in the appropriate biological fluid within the calibration range).

Calibration curves constructed for MDMA enantiomers over the ranges 0.10–80 μg/L in plasma and 0.05–6 mg/L in urine, and MDA enantiomers over the ranges 0.025–8 μg/L in plasma and 0.02–0.6 mg/L in urine were linear, generally yielding correlation coefficients ≥0.997, with limits of quantification of 0.10 μg/L and 0.05 mg/L for each MDMA enantiomer in plasma and urine, respectively, and 0.05 μg/L and 0.02 mg/L for MDA in plasma and urine. The analytical procedures showed acceptable within- and between-day variability (Table 1).

The methodology was further validated by analysis of a series of prepared plasma and urine control samples containing enantiomeric mixtures of the two analytes at three total concentrations. This validation approach is necessary because samples of biological origin will contain non-racemic mixtures of analytes as a result of stereoselectivity in metabolism and disposition. Representative precision and accuracy values obtained for the analysis of MDMA in plasma are presented in Table 2. Similar data were obtained for the analysis of MDMA in urine and MDA in both plasma and urine (data not shown). The measured enantiomeric compositions were in good agreement with the expected values at all three total concentrations examined. These data indicate that racemization of either the derivatizing agent or analytes had not occurred during the extraction and derivatization

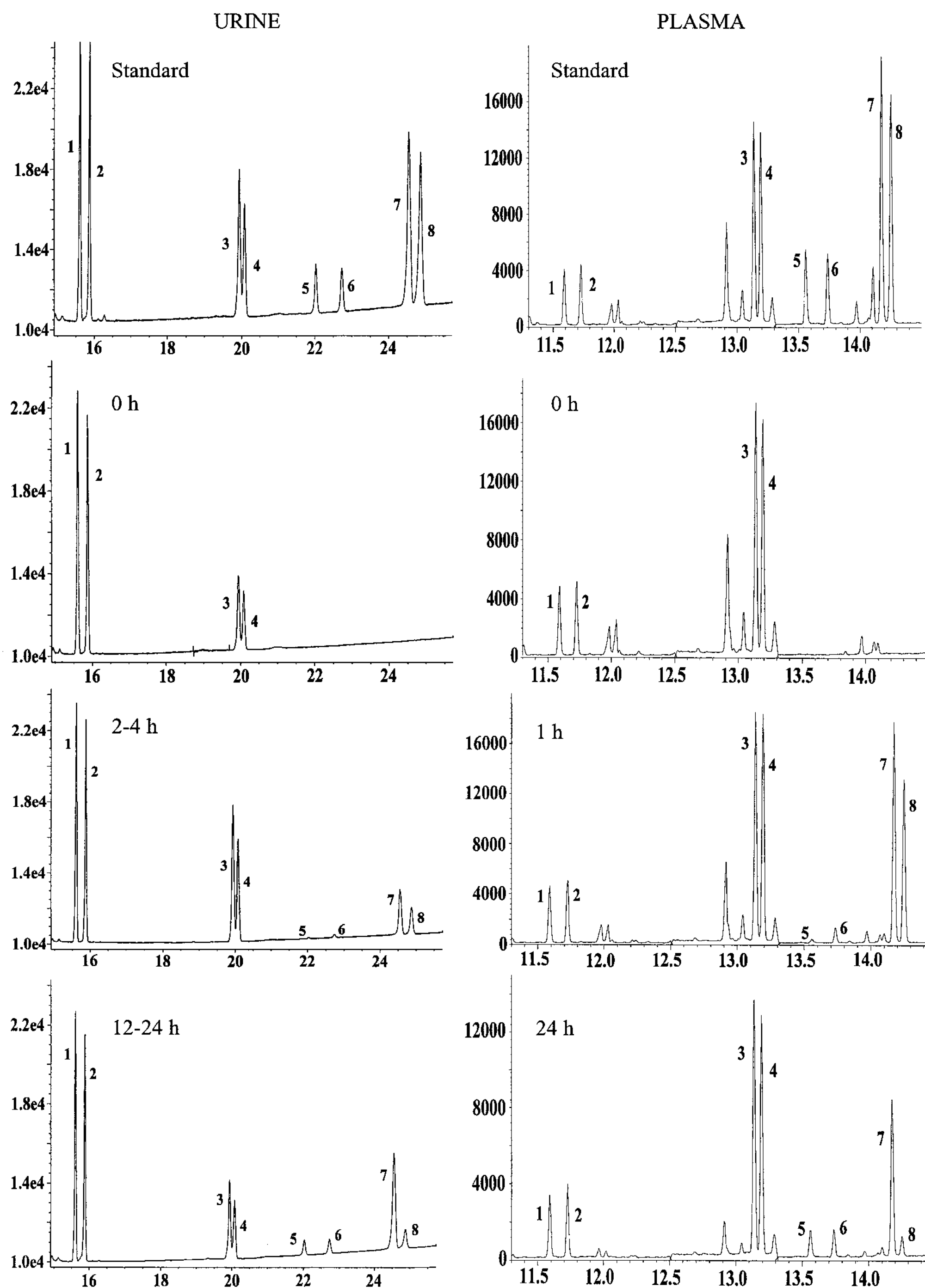

Fig. 1. GC (urine assay) and GC-MS (plasma assay) chromatograms of derivatized authentic standards following isolation from biological media, blank media to which the internal standards were added, and samples isolated post MDMA administration (detector response vs time in minutes).

Peaks: *1* and *2*, (*S*)-MTP diastereomeric amides formed on reaction of (*R*)-MTP chloride with (*R*)- and (*S*)-amphetamine; *3* and *4*, methoxyphenamine; *5* and *6*, (*R*)- and (*S*)-MDA; *7* and *8*, (*R*)- and (*S*)-MDMA. In the case of methoxyphenamine, the individual enantiomers were not available, and the elution order is unknown. Samples were collected at the times indicated on the chromatograms.

process, that stereoselectivity in derivatization had not occurred, and that increasing concentrations of the analytes do not appear to influence the enantiomeric compositions determined.

Several stereospecific methods have been reported for MDMA analysis *(14–16, 18–22)*, the majority of which use GC in combination with a chiral derivatizing agent *(14, 15, 20–22)*. The majority of these assays use *N*-trifluoroacetyl-L-prolyl chloride *(14, 20, 21)* or the corresponding N-heptafluorobutyryl derivative *(15, 22)*, reagents

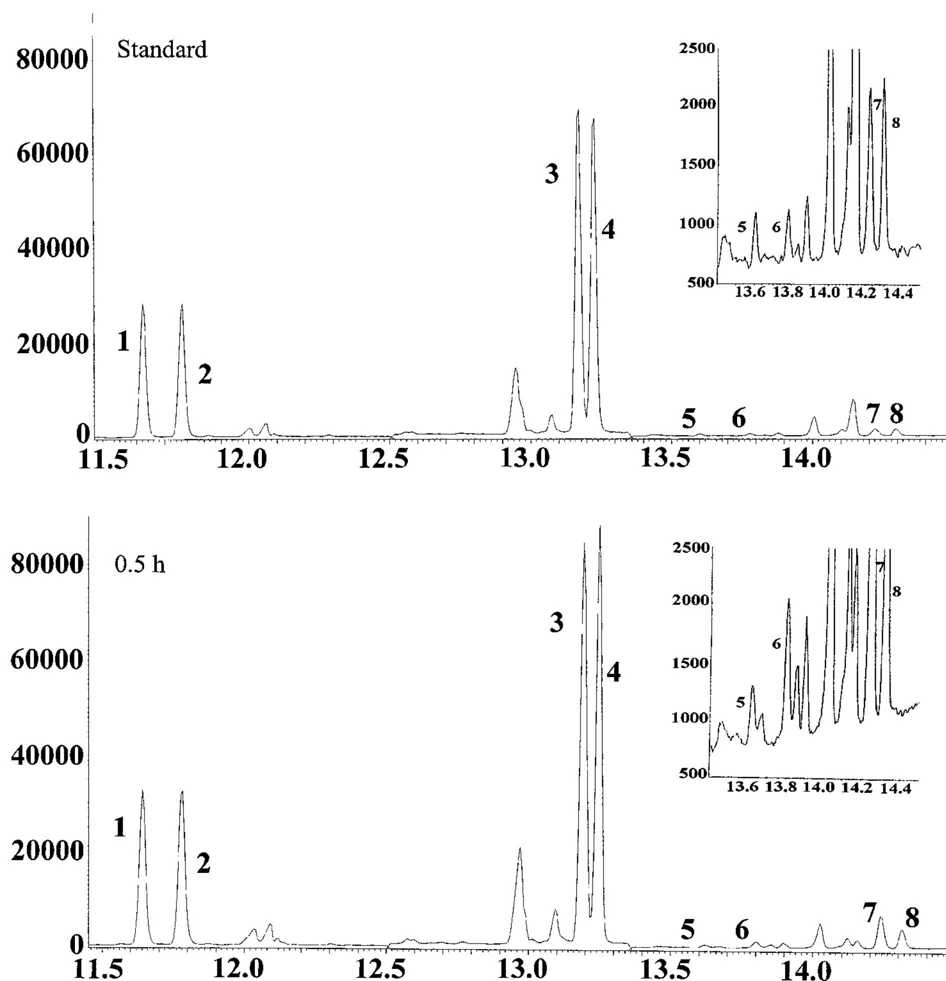

Fig. 2. GC-MS (plasma assay) chromatograms of low concentration derivatized authentic standards following isolation from plasma (*top*) and a plasma sample collected 0.5 h post MDMA administration to a healthy volunteer (*bottom*).

Peaks: *1* and *2*, (*S*)-MTP diastereomeric amides formed on reaction of (*R*)-MTP chloride with (*R*)- and (*S*)-amphetamine; *3* and *4*, methoxyphenamine; *5* and *6*, (*R*)- and (*S*)-MDA; *7* and *8*, (*R*)- and (*S*)-MDMA. In the case of methoxyphenamine, the individual enantiomers were not available, and the elution order is unknown. Single enantiomer MDMA and MDA authentic standards in plasma are at concentrations of 0.5 and 0.05 μg/L, respectively. *Insets* show an expanded view of the MDA and MDMA diastereomeric amide derivative regions of the chromatograms. *y-axis*, detector response; *x-axis*, time in minutes.

that have been noted in the literature for their lack of stereochemical purity, their instability, and unpredictable racemization *(23–27)*; therefore, some considerable care is required for the effective use of these reagents. Lim et al. *(22)* noted previously the advantages of (*R*)-MTP acid over other reagents as HCDA for the derivatization of chiral amines. However, they also reported that when they used this reagent, they were unable to obtain quantitative derivatization of racemic MDMA and three of its metabolites. Although we cannot confirm that the derivatization procedure used in the present investigation yielded quantitative conversion because we did not synthesize the corresponding amides for comparison, the validation experiments performed indicated that acceptable accuracy and precision data and reliable enantiomeric compositions were reproducibly obtained. The reasons for the differences between the two studies are by no means clear, but may be associated with the use of the acid, which requires chemical activation, rather than the acyl chloride. The reagent (*R*)-MTP chloride, used in the present investigation, is available in high enantiomeric purity, is stereochemically stable *(28)*, and with the analytes used, yields acceptable chromatographic resolution values.

STEREOSELECTIVE DISPOSITION OF MDMA

The described methodology was used to examine the enantioselective disposition of MDMA and MDA following the oral administration of the racemic drug (40 mg) to eight healthy male volunteers. The mean MDMA and MDA plasma enantiomer concentration-time profiles are presented in Fig. 3, and the pharmacokinetic parameters derived from an examination of the individual data are shown in Table 3. Semilogarithmic plots of plasma MDMA concentration vs time for two typical volunteers are presented in Fig. 4. Pharmacokinetic parameters were not calculated for one subject because of an insufficient number of data points in the elimination phase, the 24-h plasma concentrations of both MDMA enantiomers being below the minimum limit of quantification (<0.10 μg/L) in this individual.

The maximum observed plasma concentrations (C_{max}) of both MDMA enantiomers were attained within 4 h post drug administration, the mean C_{max} value for (*R*)-MDMA (33.7 ± 14.9 μg/L) being significantly greater than that of the *S*-enantiomer (21.2 ± 10.8 μg/L; P <0.001, paired *t*-test). The plasma concentrations of (*R*)-MDMA were significantly greater than those of the *S*-enantiomer in all volunteers at all time points (P <0.05, paired *t*-test), and

Table 1. Between-day accuracy and imprecision of assays for MDMA and MDA enantiomers in urine and plasma after analysis of quality-control samples.

Analyte	Enantiomeric concentration	Concentration: *R*-enantiomer Determined	*R*-enantiomer CV, %	*S*-enantiomer Determined	*S*-enantiomer CV, %
Urine, mg/L (n = 16)					
MDMA	0.25	0.31	23	0.31	25
	2.5	2.78	6.7	2.86	6.4
	5	4.97	5.8	5.06	6.0
MDA	0.025	0.026	7.5	0.027	7.6
	0.25	0.238	7.3	0.240	7.2
	0.5	0.480	8.2	0.484	9.3
Plasma, μg/L (n = 14)					
MDMA	0.25[a]	0.22	17	0.23	15
	2.5	2.6	6.3	2.3	9.0
	30	29.5	6.9	28.0	9.4
MDA	0.05[a]	0.039	16	0.045	20
	0.25	0.22	9.6	0.19	7.7
	3.0	2.67	5.4	2.33	6.8

[a] n = 8.

the mean enantiomeric ratio (*R*:*S*) of the area under the plasma concentration-time curves ($AUC_{0-\infty}$) was 2.4 ± 0.3 (n = 7). The mean (n = 7) elimination half-life ($t_{1/2}$) of (*S*)-MDMA (3.6 ± 0.9 h) was significantly shorter than that of the *R*-enantiomer (5.8 ± 2.2 h; $P < 0.01$, paired *t*-test); the more rapid elimination of the *S*-enantiomer produced a gradual progressive enrichment in the plasma content of (*R*)-MDMA with time (Fig. 3). The oral clearance of (*S*)-MDMA was significantly greater ($P < 0.01$, paired *t*-test), 2.4-fold, than that of the *R*-enantiomer. The renal clearance of both enantiomers was similar at 10.5 ± 2.9 and 10.2 ± 3.4 L/h for (*R*)- and (*S*)-MDMA, respectively, indicating that nonrenal (i.e., metabolic) clearance is the major stereoselective process. The values obtained for the volume of distribution (V_D) indicated the more extensive distribution of the more active (*S*)-MDMA ($P < 0.01$, paired *t*-test; *S*:*R* = 1.7).

In contrast to MDMA, the plasma concentrations of (*S*)-MDA exceeded those of the *R*-enantiomer in all volunteers up to 8 h post drug administration, the observed mean C_{max} of (*S*)-MDA (3.0 ± 1.1 μg/L; n = 8) being significantly greater than that of the *R*-enantiomer (1.0 ± 0.3 μg/L; $P < 0.01$, paired *t*-test) and the $AUC_{0-8\ h}$ of (*S*)-MDA being ~2.9 times that of the *R*-enantiomer.

The reduced AUC of (*S*)- compared with (*R*)-MDMA and the increased AUC of (*S*)- compared with (*R*)-MDA are indicative of the contribution of stereoselective N-demethylation to the elimination of MDMA, as has been

Table 2. Accuracy and imprecision data for the determination of a series of enantiomeric compositions of MDMA at three different total concentrations in plasma (n = 5).

Nominal concentration, μg/L *R:S*	Measured concentration, mean ± SD, μg/L *R:S*	Nominal enantiomeric composition, % *R:S*	Measured enantiomeric composition, mean ± SD, % *R:S*
Concentration, 80 μg/L			
64:16	59.7 ± 4.19:15.1 ± 1.52	80:20	79.8:20.2 ± 1.3
48:32	45.0 ± 1.77:29.9 ± 1.80	60:40	60.1:39.9 ± 1.7
32:48	30.4 ± 2.30:48.7 ± 2.14	40:60	38.4:61.6 ± 1.5
16:64	16.6 ± 0.87:71.2 ± 3.49	20:80	18.9:81.1 ± 0.7
Concentration, 20 μg/L			
16:4	16.2 ± 0.56:4.17 ± 0.21	80:20	79.5:20.5 ± 0.3
12:8	12.1 ± 0.60:7.34 ± 0.60	60:40	62.3:37.7 ± 0.8
8:12	7.53 ± 0.62:10.4 ± 0.52	40:60	41.9:58.1 ± 0.9
4:16	4.23 ± 0.23:15.7 ± 1.23	20:80	21.3:78.7 ± 1.4
Concentration, 8 μg/L			
6.4:1.6	6.60 ± 0.32:1.59 ± 0.29	80:20	80.6:19.4 ± 0.7
4.8:3.2	4.82 ± 0.28:3.05 ± 0.23	60:40	61.3:38.7 ± 1.7
3.2:4.8	3.11 ± 0.18:4.63 ± 0.22	40:60	40.2:59.8 ± 1.2
1.6:6.4	1.48 ± 0.04:5.46 ± 0.21	20:80	21.3:78.7 ± 0.8

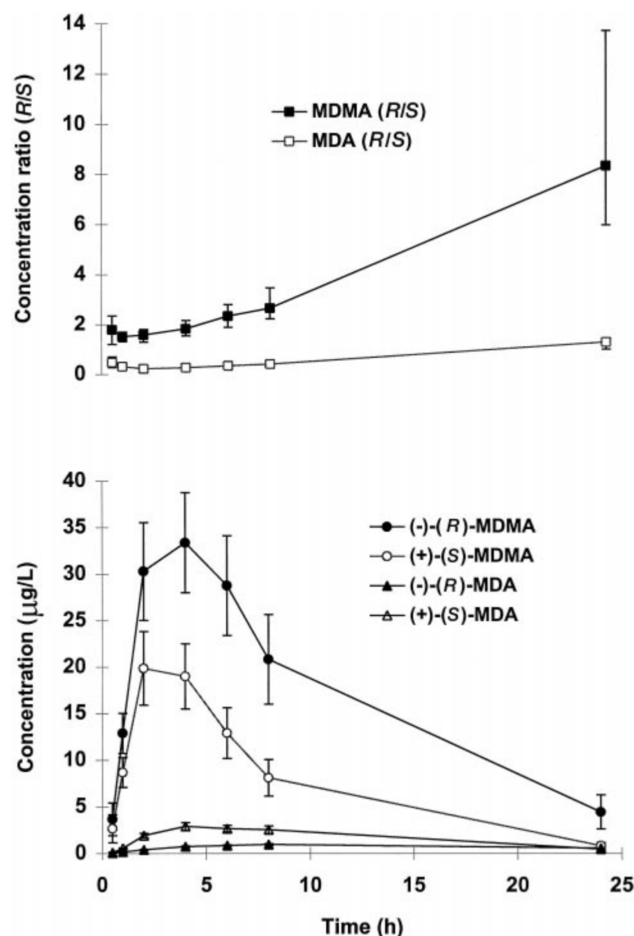

Fig. 3. Mean MDMA and MDA enantiomer plasma concentration profiles (*bottom*) and enantiomeric ratios (*R/S*; *top*) following the oral administration of racemic MDMA (40 mg) to eight healthy male volunteers.

Bars, ranges for enantiomeric composition (*top*) and SE for concentrations (*bottom*).

observed in animal studies *(13, 29)*. The time to the maximum (t_{max}) occurred between 4 and 8 h post MDMA administration (Fig. 3). The analysis of the MDA data was more limited than that of MDMA because the later t_{max} produced fewer data points during the metabolite elimination phase. The enantiomeric ratio (*R*:*S*) of MDA in plasma was found to decrease initially, reaching a minimum (0.02 ± 0.03) at 2 h, which was followed by a slow but progressive increase to 1.29 ± 0.15 at 24 h. The initial decrease presumably arises as a result of stereoselective demethylation of (*S*)-MDMA during first-pass metabolism, whereas the resulting increases are attributable to differences in the disposition of both MDMA and MDA.

The analysis of urine samples collected over the 72 h post drug administration indicated that the majority of the excreted material was recovered in the first 24 h, with ~2% of the dose being recovered in the 24- to 72-h collection period (Table 4). The urinary recovery of (*R*)-MDMA was significantly greater than that of the *S*-enantiomer, with mean values of 21.4% and 9.3% of the dose, respectively, being excreted in the first 24 h ($P < 0.01$, paired *t*-test; n = 8). Quantification of both MDMA enantiomers in the 24- to 48-h urine samples was possible for one subject: only the *R*-enantiomer could be detected in four subjects, and in only one case could (*R*)-MDMA be detected in a 48- to 72-h sample. Similarly, the majority of the recovered MDA was excreted within 24 h, the metabolite recovery being ~1% for both enantiomers. The mean urinary enantiomeric concentration ratio (*R*:*S*) for MDMA increased from 1.4 in the 0- to 2-h sample to 4.2 in the 12- to 24-h sample. In contrast, the mean urinary enantiomeric composition (*R*:*S*) of MDA were 0.48–1.2 in the 2- to 4-h and 12- to 24-h samples, respectively.

This investigation has demonstrated that MDMA undergoes extensive enantioselective disposition in humans, the more pharmacologically active *S*-enantiomer having a shorter half-life, reduced AUC, increased clearance, and undergoing more extensive distribution compared with the *R*-enantiomer. These data are similar to those observed previously following drug administration to rats *(13, 29)*. If the differences in disposition are associated primarily with stereoselective first-pass metabolism, as has been suggested from animal studies *(13)*, then variations in the hepatic extraction ratio as a result of drug interactions *(30)*, genetic polymorphism in oxidation *(31, 32)*, or disease could produce altered enantiomeric compositions in plasma, which may have toxicological implications. For example, it has been proposed that the more extensive N-demethylation of (*S*)-MDMA to (*S*)-MDA may contribute to drug toxicity *(13)*. However, it is difficult, because of legal and ethical considerations, to

Table 3. Pharmacokinetic parameters of the individual enantiomers of MDMA after the administration of the racemic drug (40 mg) to healthy male volunteers.

	C_{max}, µg/L		t_{max}, h		$t_{1/2}$, h		V_D/F, L		CL/F, L/h		CL_R/F, L/h	
Enantiomer	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
Mean[a]	33.7	21.2[b]	4	2	5.8	3.6[b]	383	595[b]	55	131[b]	10	10
SD	14.9	10.8			2.2	0.9	97	204	32	76	3	3
Maximum	56.9	41.3	4	4	10.0	5.1	532	967	109	276	13	14
Minimum	16.2	9.1	2	2	3.4	2.4	263	371	19	51	5	5

[a] Median values shown for t_{max} (not statistically tested). For C_{max} and t_{max}, n = 8, and n = 7 for the remaining variables (see text).
[b] Significant difference between enantiomers (paired *t*-test, $P < 0.01$).

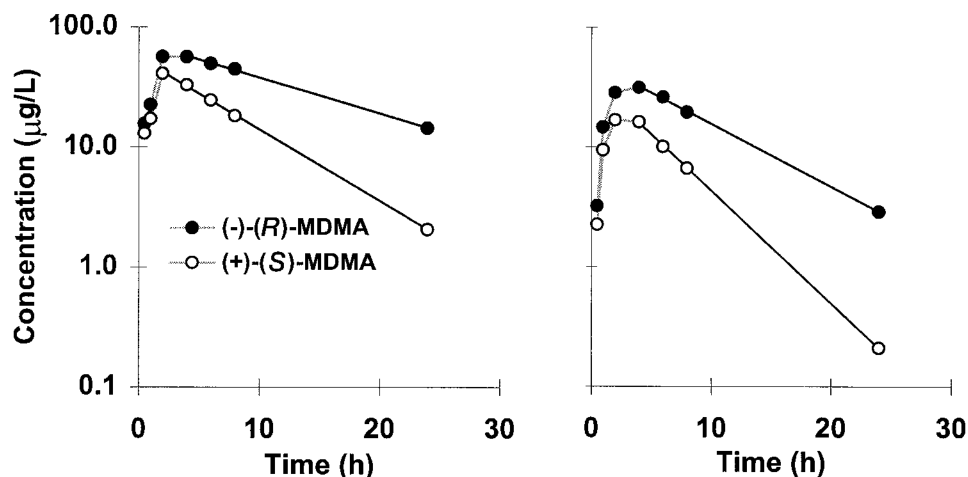

Fig. 4. Semilogarithmic plots of the plasma MDMA enantiomer concentrations following the administration of MDMA to two typical volunteers.

investigate the potential toxicity of the enantiomers of metabolites of MDMA in humans.

An alternative route of metabolism of MDMA in humans involves demethylenation, leading to the formation of HMMA *(16, 17)*, and the published data appear to support HMMA being a major metabolite. To date, only the investigation by Lanz et al. *(16)* has addressed the stereochemistry of this metabolite in urine. Because stereochemically defined standards were unavailable *(16)*, interpretation of the data was limited; however, the enantiomeric urinary recovery was reversed in the two patients examined. We currently are investigating an alternative extraction process, together with dual derivatization approaches, for the analysis and determination of the enantiomeric composition of HMMA in urine. The results of these investigations will be reported in due course.

EVIDENTIAL VALUE OF ENANTIOMERIC DISPOSITION DATA

The determination of the enantiomeric composition of an illicit drug, used as a racemate, in a biological fluid increases the "information content" of a sample and may provide evidence that an illicit material, as opposed to a prescription product, has been administered *(33)*. Determination of the stereochemical composition of the material may also provide useful pharmacokinetic information. It is frequently the case in the analysis of samples of forensic interest that the analytical toxicologist is required to draw conclusions on the basis of a minimal number of samples, frequently a single sample, with respect to the time, dose, and route of administration *(34)*. Estimation of the time elapsed between drug ingestion and sampling time is an important problem in forensic toxicology. Several approaches have been adopted in an attempt to solve this problem, including examination of drug-to-metabolite or metabolite-to-metabolite tissue/biological fluid concentration ratios with time *(34)*. When pharmaceutical preparations subject to abuse contain two or more active agents, a knowledge of the pharmacokinetic properties of the individual agents, together with the fact that the agents were administered simultaneously, facilitates the estimation of ingestion time [Fish and Tilstone, 1979, cited in Ref. *(34)*] *(35)*. However, this approach is limited by the number of commercially available combination products that are also the subject of abuse. This is not the case with substances that are administered as racemates because in such cases, two agents that frequently differ in terms of their pharmacokinetic properties are administered simultaneously at the same dose. A knowledge of the stereochemical composition of a drug, administered as a racemate, in biological media has the potential to aid in estimations of postdosing time. To the best of our knowledge, this hypothesis had not been tested previously, and therefore, we examined various mathematical models

Table 4. Urinary recovery of the enantiomers of MDMA and MDA after the oral administration of racemic MDMA (40 mg) to healthy volunteers (n = 8).

	Recovery, %					
	MDMA, 0–24 h		MDA, 0–24 h		Total recovery, 0–72 h	
	R	***S***	***R***	***S***	***R***	***S***
Mean	21.4	9.3[a]	1.0	1.4[a]	24.6	10.9[a]
SD	11.6	4.9	0.3	0.5	15.4	5.6
Maximum	38.4	15.5	1.3	1.9	53.9	19.0
Minimum	4.8	1.6	0.4	0.7	5.5	2.3

[a] Significant difference between enantiomers (paired *t*-test, $P < 0.01$).

utilizing the data obtained in the present study with MDMA.

A model could be developed on a purely theoretical basis, using the known calculated drug pharmacokinetic parameters. The problem with this approach is that a nonlinear model would result, and we have insufficient data to estimate the variables of such a model satisfactorily. We therefore adopted a more pragmatic approach and examined a variety of linear models, with time as the response, using either total or individual enantiomer drug plasma concentrations. These models were tested using multiple regression analysis. In the sequel, t is the time in hours post drug ingestion, and (R)-, (S)-, and (R,S)-MDMA are the concentrations of the individual enantiomers and total MDMA in plasma, respectively. Throughout the analysis, the data obtained at $t = 0.5$ h were excluded because in each case their inclusion produced a worse fit, presumably associated with either erratic or variable drug absorption within the group. Initial models used either total or individual enantiomer MDMA plasma concentrations as the predictors. The model developed using total MDMA plasma concentrations, i.e., (R,S)-[MDMA], was significant [$F = 7.8$ ($df = 1{,}43$), $P = 0.0077$], but the squared multiple correlation coefficient (r^2) was only 0.134. Revision of the model using the individual enantiomer concentrations produced a somewhat improved fit [$F = 7.7$ ($df = 2{,}42$), $P = 0.0015$], but, nonetheless, the r^2 value for this was only 0.267. Because these models were unsatisfactory, the next approach involved the single predictor that combined both enantiomers, namely the ratio (R:S) of the MDMA plasma concentrations. The model produced was highly significant [$F = 168$ ($df = 1{,}43$), $P < 0.00005$] and yielded a much improved r^2 value of 0.796. Logarithmic transformation of total MDMA plasma concentrations also produced a significant model [$F = 24.7$ ($df = 1{,}43$), $P < 0.00005$], but with a reduction in r^2 to 0.351. However, because of stereochemical differences in disposition, there is no a priori reason to believe that the best predictor is the simple ratio rather than a ratio of differing powers of the enantiomeric concentrations. Because:

$$\ln\{(R)\text{-}[MDMA]^a/(S)\text{-}[MDMA]^b\} = a\ln(R)\text{-}[MDMA] - b\ln(S)\text{-}[MDMA]$$

a model was fitted using the natural logarithms of the enantiomer concentrations as the predictors. Again the model was highly significant [$F = 220$ ($df = 2{,}42$), $P < 0.00005$] and gave a further improved r^2 value of 0.913. Thus, the final model fitted was:

$$t = 13.17\ln(R)\text{-}[MDMA] - 12.78\ln(S)\text{-}[MDMA] - 5.12$$

As well as being highly significant overall ($P < 0.00005$), each coefficient was also significant to the model ($P < 0.01$), i.e., both enantiomer concentrations were required. The residuals of the model were examined to check the assumptions of regression analysis, which were reasonably satisfied. Predicted times were in good agreement with actual sampling times (Fig. 5). Inevitably, there is substantial overlap between the predicted times at adjacent actual time points. This simply reflects the fact that the errors of prediction are greater than the time intervals used in the study. The lack of overlap between prediction ranges at 6-h interval separations suggests that the model is accurate enough to predict within a 6-h range. Future experiments involving more subjects and more frequent sampling may allow refinement of the model and sharper predictions.

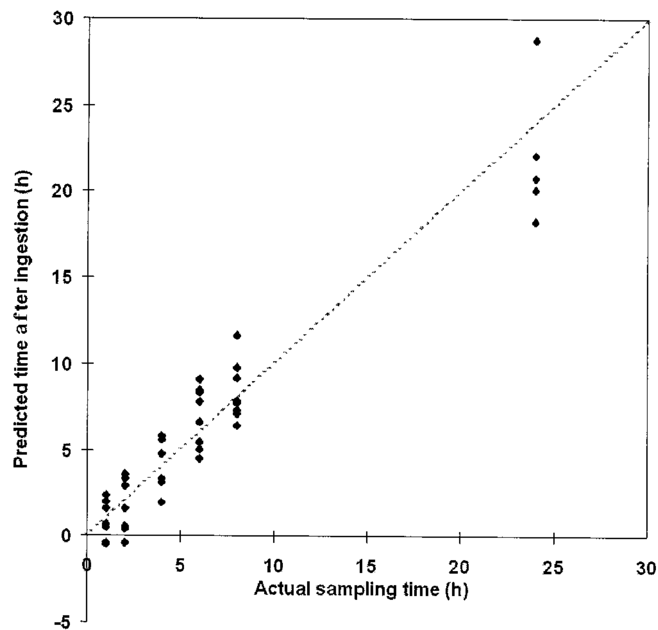

Fig. 5. Predicted time after ingestion vs actual plasma sampling time following oral administration of racemic MDMA (40 mg).

Predicted times were calculated using MDMA enantiomeric concentrations in multiple regression analysis. A line of identity (*dashed line*) is shown from 0 to 30 h.

Notwithstanding the above limitations, the correlation equations involving enantiomeric composition as a variable were in each case better than those in which concentration alone was used, irrespective of the log transformation. Further analyses would have included enantiomeric concentrations of plasma MDA and other metabolites, but the fit now seemed good enough. Because the model only applied for 1–24 h (the data were not primarily collected for this analysis) it would be interesting in future studies to examine the influence of multiple dosing and the problems associated with variable dose intervals.

In conclusion, analytical methodology suitable for the determination of the enantiomeric composition of both MDMA and MDA in plasma and urine has been developed and applied to an investigation of the disposition of MDMA in humans. To our knowledge, this is the first

report to demonstrate the stereoselective disposition of MDMA in healthy volunteers and to show the more extensive distribution and rapid clearance of the more pharmacologically active *S*-enantiomer in humans.

References

1. Nichols DE, Oberlander R. Structure-activity relationships of MDMA-like substances. Research monograph 94. Rockville, MD: National Institute on Drug Abuse, 1989:1–29.
2. Weil A. The love drug. J Psychedelic Drugs 1976;8:335–7.
3. Vereby K, Alrazi J, Jaffe JH. The complications of "Ecstasy" (MDMA). JAMA 1988;259:1649–50.
4. Climko RP, Roehrich H, Sweeney OR, Al-Razi J. Ecstasy: a review of MDMA and MDA. Int J Psychiatry Med 1986;16:359–72.
5. Steele T, McCann UD, Ricaurte GA. 3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy"): pharmacology and toxicology in animals and humans. Addiction 1994;89:539–51.
6. Milroy CM, Clark JC, Forrest ARW. Pathology and deaths associated with "ecstasy" and "eve" misuse. J Clin Pathol 1996;49: 149–53.
7. Drug Enforcement Administration. Microgram XXVIII (8). Washington, DC: Drug Enforcement Administration, 1995:235.
8. Henry JA, Fallon JK, Kicman AT, Hutt AJ, Cowan DA, Forsling M. Low dose MDMA ("Ecstasy") induces vasopressin secretion. Lancet 1998;351:1784.
9. Henry JA, Jeffreys KJ, Dawling S. Toxicity and deaths from 3,4-methylenedioxymethamphetamine ("ecstasy"). Lancet 1992;340: 384–7.
10. Dowling GP, McDonough ET III, Bost RO. "Eve" and "Ecstasy". A report of five deaths associated with the use of MDEA and MDMA. JAMA 1987;257:1615–7.
11. Green RA, Goodwin GM. Ecstasy and neurodegeneration. Br Med J 1996;312:1493–4.
12. Anderson GM III, Braun G, Braun U, Nichols DE, Shulgin AT. Absolute configuration and psychotomimetic activity. Research monograph 22. Rockville, MD: National Institute on Drug Abuse, 1978:8–15.
13. Fitzgerald RL, Blanke RV, Poklis A. Stereoselective pharmacokinetics of 3,4-methylenedioxymethamphetamine in the rat. Chirality 1990;2:241–8.
14. Moore KA, Mozayani A, Fierro MF, Poklis A. Distribution of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) stereoisomers in a fatal poisoning. Forensic Sci Int 1996;83:111–9.
15. De Boer D, Tan LP, Gorter P, Van de Wal RMA, Kettenes-van den Bosch JJ, De Bruijn EA, Maes RAA. Gas chromatographic/mass spectrometric assay for profiling the enantiomers of 3,4-methylenedioxymethamphetamine and its chiral metabolites using positive chemical ionization ion trap mass spectrometry. J Mass Spectrom 1997;32:1236–46.
16. Lanz M, Brenneisen R, Thormann W. Enantioselective determination of 3,4-methylenedioxymethamphetamine and two of its metabolites in human urine by cyclodextrin-modified capillary zone electrophoresis. Electrophoresis 1997;18:1035–43.
17. Helmlin HJ, Bracher K, Bourquin D, Vonlanthen D, Brenneisen R. Analysis of 3,4-methylenedioxymethamphetamine (MDMA) and its metabolites in plasma and urine by HPLC-DAD and GC-MS. J Anal Toxicol 1996;20:432–40.
18. Rizzi AM, Hirz R, Cladrowa-Runge S, Jonsson H. Enantiomeric separation of amphetamine, methamphetamine and ring substituted amphetamines by means of a β-cyclodextrin-chiral stationary phase. Chromatographia 1994;39:131–7.
19. Tagliaro F, Manetto G, Bellini S, Scarcella D, Smith FP, Marigo M. Simultaneous chiral separation of 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxyethylamphetamine, ephedrine, amphetamine and methamphetamine by capillary electrophoresis in uncoated and coated capillaries with native beta-cyclodextrin as the chiral selector: preliminary application to the analysis of urine and hair. Electrophoresis 1998;19:42–50.
20. Fitzgerald RL, Blanke RV, Glennon RA, Yousif MY, Rosecrans JA, Poklis A. Determination of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine enantiomers in whole blood. J Chromatogr B 1989;490:59–69.
21. Hegadoren KM, Baker GB, Coutts RT. The simultaneous separation and quantitation of the enantiomers of MDMA and MDA using gas chromatography with nitrogen-phosphorous detection. Res Commun Subst Abuse 1993;14:67–80.
22. Lim HK, Su Z, Foltz RL. Stereoselective disposition: enantioselective quantitation of 3,4-(methylenedioxy)methamphetamine and three of its metabolites by gas chromatography/electron capture negative ion chemical ionization mass spectrometry. Biol Mass Spectrom 1993;22:403–11.
23. Nichols DE, Barfknecht CF, Rusterholz DB. Asymmetric synthesis of psychotomimetic phenylisopropylamines. J Med Chem 1973; 16:480–3.
24. Gal J. Stereoisomer separations via derivatization with optically active reagents. LC-GC 1987;5:106–26.
25. Bonner WA. The adaptation of diastereomeric *S*-prolyl dipeptide derivatives to the quantitative estimation of *R*- and *S*-leucine enantiomers. J Chromatogr Sci 1972;10:159–64.
26. Payan IL, Cadilla-Perezrios R, Fisher R, Man EH. Analysis of problems encountered in the determination of amino acid enantiomeric ratios by gas chromatography. Anal Biochem 1985;149: 484–91.
27. Silber B, Riegelman S. Stereospecific assay for (−) and (+) propranolol in human and dog plasma. J Pharmacol Exp Ther 1980;215:643–8.
28. Dale JA, Dull DL, Mosher HS. α-Methoxy-α-trifluoromethylphenylacetic acid, a versatile reagent for the determination of enantiomeric composition of alcohols and amines. J Org Chem 1969;34: 2543–9.
29. Cho AK, Hiramatsu M, DiStefano EW, Chang AS, Jenden DJ. Stereochemical differences in the metabolism of 3,4-methylenedioxymethamphetamine in vivo and in vitro: a pharmacokinetic analysis. Drug Metab Dispos 1990;18:686–91.
30. Singh A. Ecstasy and Prozac. New Scientist 1995;1999:51.
31. Tucker GT, Lennard MS, Ellis SW, Woods HF, Cho AK, Lin LY, et al. The demethylation of methylenedioxymethamphetamine ("Ecstasy") by debrisoquine hydroxylase (CYP2D6). Biochem Pharmacol 1994;47:1151–6.
32. Lin LY, DiStefano EW, Schmitz DA, Hsu L, Ellis SW, Lennard MS, et al. Oxidation of methamphetamine and methylenedioxymethamphetamine by CYP2D6. Drug Metab Dispos 1997;25:1059–64.
33. Fitzgerald RL, Ramos JM Jr, Bogema SC, Poklis A. Resolution of methamphetamine stereoisomers in urine drug testing: urinary excretion of *R*-(−)-methamphetamine following use of nasal inhalers. J Anal Toxicol 1988;12:255–9.
34. Stead AH. The use of pharmacokinetics and drug-metabolism data in forensic toxicology. In: Bridges JW, Chasseaud LF, eds. Progress in drug metabolism, Vol. 9. London: Taylor & Francis, 1986:175–237.
35. Robinson AE, McDowall RD. The distribution of amylobarbitone, butobarbitone, pentobarbitone and quinalbarbitone and hydroxylated metabolites in man. J Pharm Pharmacol 1979;31:357–65.